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INSTITUTE REPORT NO. 156

FOURTEEN-DAY SUBCHRONIC ORAL TOXICITY STUDY OF
4-NITROPHENYL METHYL (PHENYL) PHOSPHINATE
IN MALE AND FEMALE RATS

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TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

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AUGUST 1983

Toxicology Series 40

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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**Fourteen-Day Subchronic Oral Toxicity Study of 4-Nitrophenyl Methyl (Phenyl)
Phosphinate in Male and Female Rats (Toxicology Series 40)--
Lewis, Hanes, Waring, Mellick, Marrs and Fruin**

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acetylcholinesterase activities were determined. Blood and urine analyses indicated no compound related effects, except slightly depressed red blood cell acetylcholinesterase activity. The only compound related effects found in the histological examinations were gross changes in the digestive system in one or more male rats at the two highest dose levels.

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ABSTRACT

A short-term multidose oral toxicity study for 4-nitrophenyl methyl (phenyl) phosphinate (MPP) was conducted as a preliminary test for a prolonged 90-day study. Four dose groups of male and female rats (1/2, 1/4, 1/8, and 1/16 of the LD₅₀) were used. Rats were dosed daily by gastric gavage for 14 days. Necropsies and histological examinations were performed on all animals. Urinalysis, standard hematological measurements and an array of serum analyses were performed. In addition, plasma, red blood cell and brain acetylcholinesterase activities were determined. Blood and urine analyses indicated no compound related effects, except slightly depressed red blood cell acetylcholinesterase activity. The only compound related effects found in the histological examinations were gross changes in the digestive system in one or more male rats at the two highest dose levels.

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PREFACE

TYPE REPORT: 14-Day Subchronic Oral Toxicity GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129

SPONSOR: US Army Medical Research and Development Command
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010

PROJECT: 35162772A875 Defense Against Chemical Agents,
WU 304 Toxicity Testing of Phosphinate Compounds,
APC TL04

GLP STUDY NUMBERS: 81034 and 81035

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATORS: Carolyn M. Lewis, MS, DAC and
CPT Martha A. Hanes, DVM, VC

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Diplomate of American College of Veterinary Pathologists
and MAJ Glen E. Marrs, DVM, MS, VC

STATISTICIAN: Virginia L. Gildengorin, PhD, DAC

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols,
raw data, retired SOPs and aliquot of the
test compound will be retained in the LAIR
Archives.

TEST SUBSTANCE: 4-nitrophenyl methyl (phenyl) phosphinate (MPP)

INCLUSIVE STUDY DATES: 5 January - 26 February 1982

OBJECTIVE: To help evaluate and characterize the potential effects of
MPP in rats when they are exposed repeatedly over a 14-day
period, to determine the dose levels for a
90-day study and possibly identify target organs.

ACKNOWLEDGMENTS

Many people contributed to the success of this study. In the Toxicology Group, we thank SSG L. White, SP5 J. Alletto, BS; SP5 L. Sauers, MS; SP4 T. Kellner, BS; SP4 L. Mullen, BS; SP4 J. Rodriguez, BS; and SP4 E. Zimmerman for their assistance in performing daily dosing and observations. In the Pathology Group, we thank CPT G. Makovec, DVM; CPT M. Langford, DVM; SSG C. Beckett, SP5 M. McKinley, BA; SP5 F. McKinley, BA; SP4 T. Loughhead, SP4 C. Dumlao, BS; SP4 M. Kostrna, L. Cote and T. Hironaga for their contribution in the collection, preparation and histological examination of tissues and in performing the hematology and urinalysis. In the Analytical Chemistry Group, we thank M. Lyons and J. Knudsen, BS, for performing the numerous biochemical analyses. A special debt of gratitude is due Claire N. Lieske, US Army Research Institute of Chemical Defense, who provided test compound, continued advice, and willing inter-agency support.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 81034 and 81035 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Studies, outlined by the Food and Drug Administration.

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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

7 June 1983

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81034 the following inspections were made:

6 Jan 82	21 Jan 82
7 Jan 82	22 Jan 82
13 Jan 82	23 Jan 82
14 Jan 82	28 Jan 82, 0930
15 Jan 82	28 Jan 82, 1500
18 Jan 82, 0810	1 Feb 82, 0900
18 Jan 82, 0830	1 Feb 82, 0930
18 Jan 82, 1000	1 Feb 82, 1000

I hereby certify that in relation to GLP study 81035 the following inspections were made:

3 Feb 82
5 Feb 82, 1400
5 Feb 82, 1609
8 Feb 82
16 Feb 82
19 Feb 82
25 Feb 82

The report and raw data for these studies were audited on 6 Jun 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the April 82 report to management and the Study Director.

N. R. Powers
NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

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The use of organophosphinate compounds as prophylactic agents in organophosphate poisoning is currently under investigation. This research was prompted by several disadvantages associated with the conventional carbamate prophylaxis. One major disadvantage is that carbamylated cholinesterases are not responsive to current oxime therapy (1). Phosphinylated cholinesterases appear to be more responsive to oximes and in preliminary prophylactic/therapy experiments in mice exposed to soman, phosphinate prophylaxis was beneficial (C.N. Lieske, et al, in presentation at 181st National Meeting of the American Chemical Society, Atlanta, GA, March 1981) (2). If further research with animals confirms that phosphinates are effective prophylactic agents eventual testing in human volunteers would be done. The U.S. Army Medical Research and Development Command Human Use Committee, in accordance with the Food and Drug Administration regulations, requires certain standard toxicological tests in animals before human testing can be performed. The present study is part of a series of toxicological testing for phosphinates.

Objective of the Study.

The objective of this study was to help evaluate and characterize the potential effects of 4-nitrophenyl methyl (phenyl) phosphinate (MPP) in rats when they are repeatedly exposed over a 14-day period, to determine the dose levels for a 90-day study and to identify target organs.

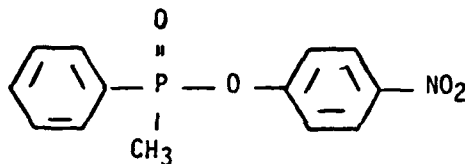
METHODS

Test Substance

Chemical name: 4-Nitrophenyl Methyl (Phenyl) Phosphinate

Lewis--2

Molecular structure: $C_{13}H_{12}NO_4P$



Additional information about the test substance appears in Appendix A.

Rationale for Selection of the Vehicle

Generally in subchronic oral toxicity studies the compound is administered by mixing it with the diet. However, due to the susceptibility of the test compound to hydrolysis in neutral or alkaline pH, this method was not used. Instead the compound was administered by oral gavage in daily doses.

Vegetable oil, historically used in oral LD_{50} studies as the carrier for water insoluble compounds, was not used in this study because of the problem of hydrolysis and the inability to verify analytically the concentration of chemicals. A carrier was formulated by the Analytical Chemistry Group (LAIR) which would solubilize the compound while stabilizing the pH and would allow spectrophotometric monitoring of hydrolysis (personal communications, P. Waring, Letterman Army Institute of Research, 15 November 1981). The carrier contained 21.5% polysorbate 80 (Tween 80), 18.5% absolute ethanol, 37.5% 50mM citrate buffer, pH 3.2, 22.5% distilled water. One gram fructose per 100 ml carrier was added to increase palatability.

Compound Preparation and Analysis

A quantity of vehicle sufficient for the entire 14-day period of dosing was prepared several days before dosing began. A new stock solution of the test compound was prepared at the beginning of each week according to LAIR SOP OP-STX-48. Aliquots of the stock solution and vehicle were pipetted into separate vials for each day of dosing to prevent contamination and then were stored in a refrigerator until needed. Just before dosing, the vials for that day were placed in a beaker of hot tap water for 15 to 30 minutes.

The stock solution and vehicle were assayed for intact and hydrolyzed phosphinate by using the spectrophotometric measurement of p-nitrophenol (LAIR SOP OP-STX-49). Samples of these were assayed before being used the first time and then each day after dosing.

Animal Data

Animal data appear in Appendix B.

Environmental Conditions

Environmental conditions are described in Appendix C.

Dosing

The dose for each animal was based on the body weight and the assigned dose group. Doses were calculated by a program on a Hewlett-Packard 98A calculator (LAIR SOP OP-ISG-8). The animals were weighed twice a week and doses were adjusted accordingly. The volume administered was kept constant at 1 ml. The doses were adjusted by varying the amount of stock solution given and by making up the difference in the volume with the additional vehicle.

Four dose levels were given to male and female rats (10 animals/sex/dose level) at 1/2, 1/4, 1/8 and 1/16 of the acute LD₅₀ for MPP each day. Table 1 shows the dosing scheme (Appendix D).⁵⁰ Each dose level was assigned two groups. One group began on a Monday and the other group began on a Thursday. This dosing scheme was necessary in order to handle all the samples for clinical and analytical chemistry and histopathology on the day of sacrifice. All animals were dosed daily at approximately 0900 hours for 14 days. The animals were not fasted overnight. An 18-gauge, 3-inch gastric gavage needle (Popper and Sons, Inc., New Hyde Park, NY 11040) was used to administer the compound by gastric intubation. This was performed without sedation or anesthesia of the animals.

Observations

Animals were checked daily for signs of illness during the quarantine period. During the exposure period animals were observed once a day in the afternoon and at 0730 hours on the day of sacrifice.

Duration of Study

The experimental phase of the study lasted for 14 days. Animals were quarantined and acclimated for 12 to 15 days before dosing began (Appendix E).

Analytical Chemistry

In Appendix F, the analytical chemistry procedures are listed.

Statistical Methods

The assignment of the dose levels to group numbers was randomized by using the RANDOM program (LAIR SOP OP-ISG-21). Animals were assigned to groups by a stratified randomization technique (LAIR SOP OP-ISG-24) available on the TOXSYS^R software (Beckman Instruments, Inc., Oceanside, CA 92054). The results from hematology and blood chemistry analyses were analyzed statistically with packaged programs available on MINITAB II^R software (3). The vehicle control group was compared with dose groups by a one-way analysis of variance. When significant differences were found a posteriori multiple comparisons were used to test for differences among means from the vehicle control group by the one-sided Dunnett's Test (4). The cage control groups were compared by a Student's t-test. Results for males and females were examined separately. LD₅₀ and slope determinations were calculated with the PROBIT program (LAIR SOP-OP-STX-51) by using the Bliss method of probit analysis (5). Survival times were analyzed by the Cutter-Ederer actuarial life table method available on the BMDP1L program of the BMDP software (6).

Changes to Original Objectives and Procedures

Some changes in the original experiment design occurred (Appendix G).

RESULTS

Survival Analysis

One of the objectives of a 14-day limit test for a subchronic toxicity study is to estimate the maximum tolerated dose which is the highest dose that the animals can survive without effect (i.e., no effect level). Ideally, for three dose levels, there should not be any deaths in the highest dose group; although, there should be signs of toxicity. In the present study, a number of deaths occurred, primarily in the highest of four dose levels for the males (Table 2).

Using the actuarial life table method with time of survival as input, the median time of survival for male rats in the highest dose group was calculated. In Table 3 are the rough estimates of the survival times for 75%, 50% (median) and 25% of the rats in this group. The results here depend upon the time intervals used. In this case, each time interval corresponds to one day. The plot for the cumulative survival function, a step function that decreases at times corresponding to the beginning of each time interval, is shown in Figure 1.

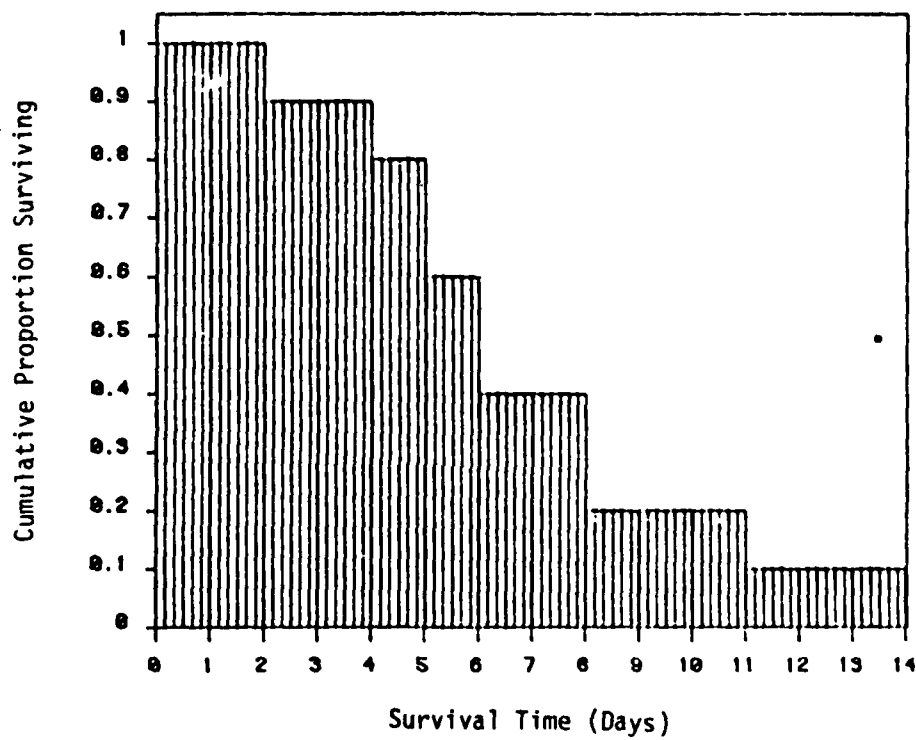


Figure 1. Plot of the Cumulative Survival Function for Male Rats Receiving 17 mg/kg/day.

A 14-dose LD₅₀ (mg/kg/day) was calculated for the males by probit analysis. These values are given in Table 4.

Clinical Signs

Animals were dosed in the morning and observed about six hours later. Animals were observed undisturbed in cages, outside of cages and after returning to cages. Unscheduled observations of animals exhibiting severe signs of toxicity after dosing were also recorded.

One advantage a subchronic toxicity study has over an acute toxicity study is that most animals survive and therefore, the incidence of signs recorded more often suggests a dose-response relationship. In both male and female rats collapse or splayleg, changes in respiratory rate or depth, tremors, twitching, gasping, excessive salivation, piloerection or rough coat, loss of equilibrium, humpback (hunched posture), red material or stain on anterior portion of body (probably harderian gland secretion), orange material or stain on anterior portion of body and yellow material or stain on posterior section of body appeared to be dose-related. Some of these signs were more common in males; such as, gasping, salivation, collapse or splayleg. Twitching was recorded only in males. Changes in respiratory rate, tremors, and piloerection were more common in females. Two signs that we observed only in females were changes in respiratory depth and rough coat.

There were a few other signs we observed almost exclusively in the high dose groups. Most were seen in both sexes. These include opisthotonos, head extension, head weaving, head tossing, head tilt, chewing, retching, vomiting, circling, staggering, weaving, cyanosis, decreased pupil size, "bugged" eyes, increased palpebral opening, and unresponsiveness.

There were several other signs that were seen frequently, but the incidence did not suggest a dose-response relationship. Among these were sound production (in females only), sluggishness, inactivity, irritability, aggressiveness, and excitability.

When clinical signs appeared, the onset was fairly soon after dosing. After completion of the dosing procedure and by the time scheduled observations were conducted, many of the clinical signs had disappeared. Consequently, the full gamut of toxicity signs exhibited by each animal was probably not recorded and therefore, the incidence of signs recorded is not necessarily representative of the actual incidence. For this reason and the fact that the number of animals showing a specific sign was never large, statistical analysis of the dose-response relationship was not done. Despite inherent problems with statistical analysis of clinical signs, a subjective interpretation based on recorded observations was considered valuable.

Analytical Chemistry

The effect of MPP on the level of several serum electrolytes, various biochemical components and the activity of several enzymes in serum was examined. In addition, acetylcholinesterase activity was measured in plasma, red blood cells and brain tissue. The mean and standard deviation for each dose group for these parameters are shown in Tables 5 through 8. The vehicle control and dose groups were compared by analysis of variance. The cage and vehicle control groups were compared by using the Student's t-test.

No significant differences were found in the serum electrolyte levels in either sex when the vehicle control and dose groups were compared or when the vehicle and cage control groups were compared. This was also true for the biochemical constituents analyzed in serum, except the creatinine and total protein levels for male rats in the cage control group when compared to the vehicle control group.

A few significant differences occurred in enzyme levels. Lactate dehydrogenase (LDH) activity was slightly higher in the vehicle control females. This difference was not seen in the males. Another significant difference was found in the red blood cell (RBC) acetylcholinesterase activity. The vehicle control groups had significantly higher RBC acetylcholinesterase than all the dose groups for both male and females. The RBC acetylcholinesterase in the vehicle control group was also significantly higher than the cage control group for males. There was no difference in the RBC acetylcholinesterase for the two control groups for the females. There appears to be a sex-related difference in the normal plasma cholinesterase levels if one compares the cage control group for both sexes. Female rats in the cage control group had significantly higher plasma cholinesterase activity than males in that group.

The statistician's statement appears in Appendix H.

Pathology

The pathology report appears in Appendix I.

DISCUSSION

When male and female rats received half the single dose LD₅₀ concentration in daily doses for 14 days, only one male rat survived while nine female rats survived. Some investigators have suggested that the difference in lethality of a compound with single and repeated exposures is an indication of the degree of accumulation of a compound. Hayes (7) proposed the use of a "chronicity factor" which was equal to the 1-dose LD₅₀ (mg/kg) divided by the 90-dose LD₅₀ (mg/kg/day). Kagan and Stankevich (8) suggested the use of a

"cumulation coefficient," K_{cum} , which is the ratio of the summated median lethal dose given in a subchronic study to the single dose LD_{50} . If one applies these formula to the male rats in our 14-day subchronic study of MPP, we get results which suggest the compound is accumulating to some extent. While there is insufficient data on the females, it would appear that there is not much difference for females in the lethality of MPP with single and repeated exposures. One might conclude then, that the compound accumulates more readily in males than females. However, if one compares the dose level in the 14-day subchronic study for males and females where no more than 10% of the rats died, the two levels are roughly equivalent (males = 8.50 mg/kg versus females = 6.25 mg/kg). In this case, the difference in the ratio of the single and repeated exposure LD_{50} values in rats is due primarily to differences in the single exposure LD_{50} . Therefore, to conclude that there is a sex-related difference in accumulation of MPP does not seem valid. More likely, there is simply a sex-related difference in the response to single exposures to MPP.

The type of clinical signs observed in the 14-day subchronic study of MPP did not differ greatly from those reported in the acute study (9). Nearly all signs seen could be attributed to effects of MPP on the nervous system. Some of the more frequent signs were changes in respiration, tremors, twitching, gasping, excessive salivation, loss of equilibrium and collapse. The main difference between the acute and 14-day subchronic studies was in the frequency of the clinical signs since the rats were being exposed repeatedly and at lower levels where more animals survived. There were differences between the sexes in the incidence of some signs. A higher incidence of some clinical signs in males is not surprising since they appeared to be more sensitive to repeated doses of MPP when compared to a single dose. Why some signs were more frequent in females is harder to explain. Perhaps they are signs exhibited more often with lower doses or there could be a sex related difference in their symptomatic response to the MPP. There were a few clinical signs, such as sound production, sluggishness and irritability, that showed no dose relationship. Some of these signs are probably from being dosed every day which was not a pleasant experience for the animals. Others may be related to some side-effects of the vehicle. In fact, some could be compound related. However, there is not sufficient information at this point to determine the causes.

Only a few significant differences were seen in the clinical chemistry results for blood and tissues. The cage control group for male rats had significantly lower creatinine and total protein levels in serum than the vehicle control group. No statistical differences were found between control groups in females for either creatinine or total proteins. The differences in these parameters between the male control groups could be attributed to some effect the vehicle has on these constituents and males were more susceptible. These differences

could also be statistical aberrations. At this point there is insufficient information to determine whether these differences have any clinical significance. However, the male vehicle control values were still within the normal ranges reported in the literature (10).

Female rats in the vehicle control group had significantly higher serum LDH levels than the dose groups. The clinical significance of this difference is questionable because it is opposite of what one might expect if the test compound had some adverse effect on tissues, and the values for the vehicle control group were still within the normal ranges (10).

The RBC acetylcholinesterase levels were lower in all dose groups for males and females when compared to their respective vehicle control groups. This difference was the only clearly compound related difference found in the biochemical analyses. In the males, the cage control group also had significantly lower RBC acetylcholinesterase activity than the vehicle control group. The clinical significance of this statistical difference found in males is uncertain. The vehicle could be enhancing acetylcholinesterase activity somehow and the males were particularly susceptible to this effect or this difference could merely be a statistical aberration. This difference was not seen in the females.

The toxicological significance of the inhibition of RBC acetylcholinesterase by MPP is not clear. This inhibition may or may not be related to the lethality of MPP. The function of acetylcholinesterase in the red blood cell, if any, is not known, but does not appear to be related to neural transmission. Whether this inhibition could have been the result of the repeated exposures is also not known. If cholinesterase is not completely reactivated after 24 hours, repeated daily exposures might then result in higher residual inhibition after each exposure. The increased number of deaths after four or more doses in the highest dose group for males would support this theory. However, further research is needed to determine if and where MPP is actually accumulating.

While not a compound related difference, the higher plasma cholinesterase levels in females is interesting. The cholinesterase in plasma apparently varies slightly from that in the red blood cell (11). The function of cholinesterase in plasma is not known. It may be that the difference in the plasma cholinesterase levels is related to the difference in response of the sexes to MPP. One would think higher plasma cholinesterase would increase the females' ability to survive exposure to MPP. However, if the plasma cholinesterase played a role in the transportation of the MPP to target organs, higher plasma cholinesterase levels could result in increased sensitivity to the toxic effects of MPP. If the primary lesion were known, perhaps one could determine the reason for the sex-related difference in

response to MPP. Knowing the reason for the difference between sexes in response to MPP might also help in determining the best approach for prophylaxis with organophosphate poisoning.

No differences in brain acetylcholinesterase levels were seen. The lack of inhibition suggests that the brain may not be a primary target in the lethality of MPP. Inhibition of the peripheral acetylcholinesterase in the diaphragm could be the primary lesion with MPP as it is with soman poisoning (12). More information is needed so that adequate monitoring of the toxic effects of MPP can be done in a 90-day subchronic study.

Pathological examination revealed only a few compound related effects. The deaths of male rats in the top three dose groups were attributed to the toxic effects of the compound and indicate a dose-related response. No compound related deaths occurred in the females, although one rat died from trauma caused during dosing. Gross pathological examination revealed only changes in the digestive system in a few animals in the highest dose groups. These changes in the digestive system were attributed to an irritant effect of the compound. Microscopic examination of tissues revealed no compound-related lesions in either males or females. However, hematologic examination revealed a slight lowering of RBC count, hemoglobin, hematocrit and platelet count in the highest female dose group when compared to the female vehicle control group. The platelet count was also significantly lower for the lowest female dose group when compared to the female vehicle control group. No compound related differences in the hematologic data were found in the male rats although they received higher doses and some males had died from the toxic effects of compound. Urine specimens, collected in metabolic cages, were not suitable for examination due to fecal contamination and spillage from water bottles. For these reasons, no attempts were made to evaluate urinalysis data.

CONCLUSION

When given daily doses of MPP for 14 days, the highest dose level tolerated with no more than 10% mortality was 8.50 mg/kg/day for male rats and 6.25 mg/kg/day for female rats. Blood analyses revealed only a few compound related effects. RBC acetylcholinesterase activity was slightly depressed in all dose groups for males and females. The RBC count, hemoglobin, hematocrit and platelet count in the highest female dose group (6.25 mg/kg) were slightly lower than the vehicle control group. The only compound-related effects found in the histological examinations were gross changes in the digestive system of several male rats at the two highest dose levels.

RECOMMENDATIONS

More short-term studies are probably needed before a 90-day subchronic study is conducted. First, the primary cause of death should be determined. We suggest an acute metabolic study should be conducted in which samples of plasma, red blood cells, brain, heart and diaphragm are taken at various time intervals when lethal and sublethal doses of MPP are administered. Not only would this provide information regarding the cause of death, but it also would provide more information regarding the inhibition and reactivation of cholinesterase after a single exposure to MPP. Based on our results the cholinesterase levels need to be followed longer than 24 hours.

Information regarding accumulation and tolerance of MPP after repeated exposures is needed. Specifically, the pharmacokinetics of ¹⁴C-labelled compound could provide information regarding the accumulation of MPP with repeated exposures. To test tolerance of MPP, rats could be exposed to low levels of the compound and then challenged with lethal doses similar to those used in the LD₅₀ study.

If a 90-day multi-dose study is conducted, the observation should be scheduled about an hour after dosing. Hopefully, observations made at this time would reflect the most severe signs of toxicity that occur. One problem with conducting only one observation at that time is that the results can be misleading since they do not reflect the disappearance of many signs several hours later. However, it is not possible to record the onset and disappearance of many signs without doing observations several times a day. On a long term study frequent observations may become too labor intensive to be practical. If one has to choose, noting the presence of the most severe clinical signs is more useful than the least severe ones.

Finally, while daily oral intubation was acceptable for a two-week study, it is probably not advisable for a 90-day study. Towards the end of our study the animals were becoming harder to handle during dosing. Not only did this increase the time it took to dose the animals, but it also increased the chance of injury to the animal. With a 90-day multi-dose study, this becomes a significant problem because the number of animals lost due to trauma would increase. The compound was given by oral intubation because of concern about the susceptibility of the compound to hydrolysis. If the compound were microencapsulated, one could then mix it with the diet and be fairly confident of its stability.

Incorporation of the compound into the diet would unfortunately require another limit test since blood levels would not get as high when it is ingested over a longer period of time. Also the presence of Tween 80 in the vehicle used in the present study may have affected absorption of the compound. Incorporating the compound in the diet would make the time of observation less critical.

REFERENCES

1. Natoff IL, Reiff B. Effect of oximes on the acute toxicity of anticholinesterase carbamate. *Toxicol Appl Pharmacol* 1973; 25:569-575.
2. Lieske CN, Clark JH, Meyer HF, Lowe JR. Spontaneous and induced reactivation of acetylcholinesterase inhibited by three organophosphinates. *Pestic Biochem Physiol* 1980; 13:205-212.
3. Ryan TA Jr, Joiner BL, Ryan BF. Minitab II reference manual. University Park: Pennsylvania State University, 1978.
4. Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill Book Co., 1971:201-204.
5. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.
6. Dixon WJ, ed. BMDP statistical software. Berkeley: University of California Press, 1981:555-573.
7. Hayes WJ Jr. The 90-dose LD₅₀ and a chronicity factor as measure of toxicity. *Toxicol Appl Pharmacol* 1967; 11:327.
8. Kagan YS, Stankevich VV. Cited In: Filou VA, Goluber AA, Liublina EI, Tolokonseu NA. Quantitative Toxicology (English Language Edition). New York: John Wiley & Sons, Inc., 1979:252-269.
9. Kellner TP, Hanes MA, Fruin JT. Acute oral toxicity potential of 4-nitrophenyl methyl phenyl phosphinate. Toxicology Series 35. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1982. Institute Report No. 128.
10. Laird CW. Clinical pathology: blood chemistry. In: Melby EC, Altman NH, eds. CRC handbook of laboratory animal science. Vol II. Cleveland: CRC Press, 1974:345-436.
11. Rosenberry TL. Acetylcholinesterase. *Adv Enzymol* 1975; 43:103-218.
12. Clement JG. Toxicology and pharmacology of dispyridinum oximes - insight into the mechanisms of action vs soman poisoning in vivo. *Fund Appl Toxicol* 1981; 1:193-202.

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APPENDICES

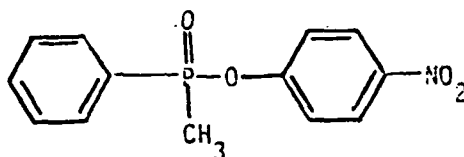
Lewis--14

TEST SUBSTANCE

1. Chemical name: 4-Nitrophenyl Methyl (Phenyl) Phosphinate

Chemical Abstract Service Registry No.: None

Molecular structure: $C_{13}H_{12}NO_4P$



Molecular weight: 277.2

pH: N/A

Physical state/color: White, fluffy crystals

Melting point: 85-86 C

Compound density: Unknown

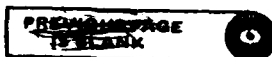
Stability: Unstable at neutral and basic pH. Stable in acidified cocktail.

Contaminants: None detected.

Manufacturer: Ash Stevens Inc. 5861 John C. Lodge Freeway
Detroit, Michigan 48202

Manufacturer Lot No: MP-07-29, 5 October 1981

Analysis (by manufacturer): Calculated for $C_{13}H_{12}NO_4P$

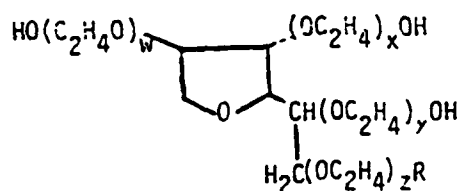


<u>Calculated Percent by Weight</u>		<u>Found</u>
C	56.33	56.17
H	4.36	4.28
N	5.05	5.14
P	11.17	11.25

2. Chemical name: Polysorbate 80 (Tween 80)

Chemical Abstract Service Registry No.: 9005-65-6

Molecular structure:



(Sum of w,x,y,z is 20;

R is (C₁₇H₃₃)COO)

Molecular weight: 1307

pH: 5-7 in 5% aqueous solution

Physical state/color: Liquid, viscous; amber-colored

Stability: Stable at room temperature

Manufacturer: Fisher Scientific Co.,
Fairlawn, New Jersey 07410

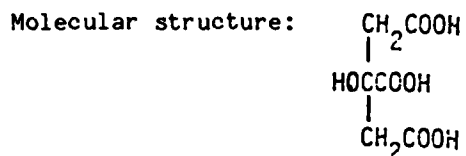
Manufacturer Lot No: 713137

Published Toxicity Data:

Considered to be pharmacologically inert and is commonly used for dispersing insoluble drugs for oral administration particularly in chronic toxicity studies in experimental data.

3. Chemical name: Citric Acid, monohydrate

Chemical Abstract Service Registry No.: 77-92-9



Molecular weight: 192.12

pH: 0.1N solution = 2.2

Physical state/color: Brachydomatic crystals; white

Melting point: Softens at 75 C and melts at 100 C

Compound Specific Gravity: 50% of aqueous solution = 1.2204

Stability: Stable at room temperatures

Contaminants: Less than 0.3%

Manufacturer: J.T. Baker Chemical Co.
Phillipsburg, New Jersey 08865

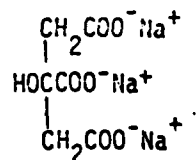
Manufacturer Lot No: 35444

Published Toxicity Data: LD₅₀ in rats (I.P.) 975 mg/kg.

4. Chemical name: Sodium Citrate

Chemical Abstract Service Registry No.: None

Molecular structure:



Molecular weight: 258 (dihydrate = 294)

pH: 5% aqueous solution at 25 C = 8.8

Physical state/color: Dihydrate, white, odorless crystals

Melting point: Becomes anhydrous at 150 C

Compound density: $D_4^{20} = 1.1492$

Stability: Stable in air at room temperature

Contaminants: Less than 0.3%

Manufacturer: J.T. Baker Chemical Co.
 Philipsburg, New Jersey 08865

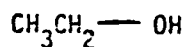
Manufacturer Lot No: 31482

Published Toxicity Data: LD₅₀ in rats (I.P. administration)
 is 6.0 moles/kg (1.5 g/kg).

5. Chemical name: Ethanol, anhydrous

Chemical Abstract Service Registry No.: 64-17-5

Molecular structure:



Molecular weight: 46

pH: N/A

Physical state/color: Clear, colorless, very mobile, flammable,
 liquid; pleasant odor; burning taste.

Boiling point: 78.5 C

Compound Specific Gravity: 1.361 g/ml

Stability: Stable if stored properly in a sealed container at
 room temperature. will absorb water rapidly from air
 if not stored properly.

Contaminants: Unknown

Manufacturer: U.S. Industrial Chemicals,
Tuscola, Illinois 61953

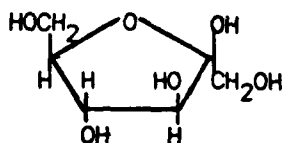
Manufacturer Lot No: 205

Published Toxicity Data: LD_{50} in rats = 13.7 g/kg

6. Chemical name: D(-) Fructose

Chemical Abstract Service Registry No.: 57-48-7

Molecular structure: $C_6H_{12}O_6$



Molecular weight: 277.2

pH: Unknown

Physical state/color: Crystalline; white

Melting point: 103-105 C (decomposes)

Compound density: $D_4^{20} = 1.60$

Stability: Unknown

Contaminants: Less than 0.05% glucose

Manufacturer: Sigma Chemical Co.
P.O. Box 14508
St. Louis, Missouri 63178

Manufacturer Lot No: 35C - 0198

Published Toxicity Data: LD_{50} in rabbits 15 g/kg (i.v.)

APPENDIX A (Concluded)

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ANIMAL DATA

Species: Rattus norvegicus

Strain: Sprague-Dawley

Source: Charles River Breeding Laboratories
Kingston, NY 12401

Sex: Male and female

Age: Males, 7 weeks at receipt
Females, 9 weeks at receipt

Method of Randomization: TOXSYS^R Animal Allocation Program

Animals in each group: 20 animals, 10 males and 10 females

Condition of animals at start of study: Normal

Body weight range: 153-203 g at receipt
Males: 232-292 g at first dosing
Females: 179-225 g, at first dosing.

Identification procedures: Ear tag (SOP OP-ARG-1)

Pretest conditioning:

- a. Animals quarantined for at least one week. (Males, 6-15 Jan 1982 and females, 27 Jan through 5 Feb 1982).
- b. Animals predosed acclimated with 1 ml of water daily. (Males 11-15 Jan 1982 and females 1-5 Feb 1982).

Justification: The Sprague-Dawley rat is a proven sensitive mammalian model for oral LD₅₀ determination and subchronic toxicity studies. The Sprague-Dawley rat from Charles River most closely resembles the response of the "Edgewood" rat to a number of test criteria.

APPENDIX B

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ENVIRONMENTAL CONDITIONS

Caging: Number per cage = 1; type of cage = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralson Purina Rodent Diet #5002, ad lib. Lot number NOV05811C

Water: Central line to cage battery

Temperature: 76 ± 4 F (Males); 75 ± 8 F (Females)

Humidity: $45 \pm 5\%$ (Males); $40 \pm 10\%$ (Females)

Photoperiod: 0600-2000 hours per day (light 14 hours)

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APPENDIX C

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TABLE 1

Dosing Scheme for 14-Day Subchronic Toxicity of MPP*

MALES		FEMALES	
Concentration (mg/kg/day)	Group No.	Concentration (mg/kg/day)	Group No.
Cage control (0)	3, 9	Cage control	1, 11
Vehicle control (0)	4, 7	Vehicle control	5, 12
2.12	5, 10	0.78	6, 8
4.25	6, 12	1.56	4, 7
8.50	1, 11	3.12	2, 9
17.00	2, 8	6.25	3, 10

*MPP = 4-nitrophenyl methyl (phenyl) phosphinate

TABLE 2

MPP* Related Deaths by Group

MALES		FEMALES	
Concentration (mg/kg/day)	Deaths/ Group Total	Concentration (mg/kg/day)	Deaths/ Group Total
Cage Control	0/10	Cage Control	0/10
Vehicle Control	0/10	Vehicle Control	0/9†
2.12	0/10	0.78	0/10
4.25	0/10	1.56	0/10
8.50	1/10	3.12	0/10
17.00	9/10	6.25	1/10

*MPP = 4-nitrophenyl methyl (phenyl) phosphinate

†D8100909 removed from study due to severe urinary tract infection which was discovered at necropsy.

APPENDIX D (Cont)

TABLE 3

Estimates of the Quantiles of Survival Function
for Male Rats Receiving 17 mg MPP^a/kg/day

Quantile	Estimate (days)	Standard Error
75th	4.25	0.68
50th (median)	5.50	0.79
25th	7.75	0.68

^aMPP = 4-nitrophenyl methyl (phenyl) phosphinate

TABLE 4

Lethal Dose Concentrations of MPP^a in Male Rats

Percentile	Dose (mg/kg/day)	95% Confidence Interval (mg/kg/day)
1st	3.4	1.3 - 9.3
50th	11.0	8.0 - 15.2
95th	25.1	10.1 - 58.4

^aMPP = 4-nitrophenyl methyl (phenyl) phosphinate

TABLE 5

Effects of 14-Day Subchronic Oral Doses of MPP* on Electrolyte Levels in Serum

	Calcium (mg/dl)		Potassium (mEq/L)		Chloride (mEq/L)		Magnesium (mg/dl)	
MALES								
Untreated Controls (n=10)	9.7	1.0 [†]	6.94	0.70	109	3	2.65	0.22
Vehicle Controls (n=10)	10.0	0.9	6.87	0.40	111	4	2.73	0.27
2.12 mg/kg (n=9)	9.9	0.9	7.08	1.05	110	3	2.58	0.19
4.25 mg/kg (n=9)	9.9	1.0	6.84	0.77	109	3	2.72	0.12
8.50 mg/kg (n=9)	10.1	0.9	6.76	0.71	111	4	2.56	0.11
17.00 mg/kg (n=1)	—		—		—		—	
FEMALES								
Untreated Controls (n=10)	11.1	0.7	8.35	0.86	101	2	3.09	0.36
Vehicle Controls (n=9)	11.4	0.5	7.98	0.52	101	2	3.11	0.25
0.78 mg/kg (n=10)	11.4	0.5	8.43	1.91	101	4	3.19	0.52
1.56 mg/kg (n=10)	11.4	0.5	7.94	0.58	101	2	3.17	0.24
3.12 mg/kg (n=10)	11.3	0.6	7.91	1.14	103	3	3.14	0.24
6.25 mg/kg (n=9)	11.4	0.4	7.76	1.00	103	3	2.91	0.13

*MPP = 4-nitrophenyl methyl (phenyl) phosphinate

†Mean ± standard deviation

APPENDIX D (Cont)

TABLE 6
Effects of MPP on Biochemical Constituents of Serum

	Glucose (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)	Uric Acid (mg/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Albumin/ Globulin Ratio	Total Protein (gm/dl)
MALES									
Untreated Controls (n=10)	153 ± 33 [†]	47.8 ± 8.4	0.47 ± 0.03 [†]	15.0 ± 1.6	1.93 ± 0.31	4.28 ± 0.38	2.34 ± 0.31	1.87 ± 0.43	6.62 ± 0.37 [†]
Vehicle Controls (n=10)	158 ± 28	52.5 ± 9.2	0.52 ± 0.05	16.0 ± 2.3	2.22 ± 0.44	4.47 ± 0.16	2.56 ± 0.29	1.77 ± 0.25	7.03 ± 0.24
2.12 mg/kg (n=9)	173 ± 20	40.6 ± 12.7	0.48 ± 0.02	15.2 ± 2.8	2.06 ± 0.41	4.56 ± 0.21	2.50 ± 0.29	1.84 ± 0.22	7.06 ± 0.35
4.25 mg/kg (n=9)	172 ± 24	46.5 ± 13.5	0.50 ± 0.04	15.5 ± 1.3	2.08 ± 0.34	4.57 ± 0.33	2.48 ± 0.33	1.89 ± 0.38	7.04 ± 0.30
8.50 mg/kg (n=9)	157 ± 33	50.8 ± 15.8	0.48 ± 0.02	16.7 ± 2.1	1.93 ± 0.23	4.46 ± 0.25	2.27 ± 0.27	2.00 ± 0.32	6.72 ± 0.34
17.00 mg/kg (n=1)	—	—	—	—	—	—	—	—	—
FEMALES									
Untreated Controls (n=10)	185 ± 34	57.1 ± 18.3	0.53 ± 0.07	16.0 ± 2.1	1.98 ± 0.40	5.28 ± 0.29	2.11 ± 0.41	2.58 ± 0.47	7.39 ± 0.36
Vehicle Controls (n=9)	187 ± 24	66.8 ± 14.5	0.52 ± 0.07	16.0 ± 2.6	1.92 ± 0.42	5.40 ± 0.60	2.25 ± 0.75	2.86 ± 1.12	7.58 ± 0.29
0.78 mg/kg (n=10)	184 ± 46	57.9 ± 11.2 [§]	0.53 ± 0.10	17.2 ± 2.9	2.06 ± 0.79	5.30 ± 0.64	2.10 ± 0.55	2.67 ± 1.11	7.55 ± 0.48
1.56 mg/kg (n=10)	172 ± 23	66.3 ± 29.9	0.54 ± 0.05	16.6 ± 2.0	1.95 ± 0.42	5.60 ± 0.31	2.01 ± 0.71	3.13 ± 1.19	7.61 ± 0.60
3.12 mg/kg (n=10)	186 ± 28	57.5 ± 14.7 [§]	0.55 ± 0.07	16.6 ± 2.8	1.91 ± 0.32 [§]	5.39 ± 0.38	2.08 ± 0.44	2.76 ± 0.91	7.47 ± 0.29
6.25 mg/kg (n=9)	189 ± 35	65.8 ± 15.0	0.52 ± 0.05	14.8 ± 2.1	1.56 ± 0.43	5.48 ± 0.53	1.79 ± 0.41	3.24 ± 0.89	7.27 ± 0.25

* MPP = 4-nitrophenyl methyl (phenyl) phosphinate

[†] Mean ± standard deviation

[‡] Significantly lower than the vehicle control group when compared by Student's t-test (p < .05)

[§] n = 9

TABLE 7

Effect of MPP^a on Enzymatic Activity in Serum

	Aspartate Amino- transferase (I.U.)	Alanine Amino- transferase (I.U.)	Lactate Dehydro- genase (I.U.)	Creatine Phospho- kinase (I.U.)
MALES				
Untreated Controls (n=10)	63.3 ± 9.8 [†]	27.0 ± 6.9	205 ± 221	61.3 ± 52.9
Vehicle Controls (n=10)	59.9 ± 8.4	23.8 ± 2.4	98 ± 42	63.1 ± 63.3
2.12 mg/kg (n=9)	62.4 ± 11.8	25.8 ± 7.2	102 ± 41	42.2 ± 22.0
4.25 mg/kg (n=9)	61.5 ± 9.0	24.6 ± 3.5	157 ± 94	58.0 ± 29.3
8.50 mg/kg (n=9)	65.2 ± 14.8	26.1 ± 2.3	163 ± 227	51.3 ± 31.2
17.00 mg/kg (n=1)	—	—	—	—
FEMALES				
Untreated Controls (n=10)	91.1 ± 22.1	30.8 ± 5.0	204 ± 121	92.1 ± 46.9
Vehicle Controls (n=9)	92.1 ± 39.7	30.9 ± 17.5	244 ± 108 [†]	109.3 ± 43.6
0.78 mg/kg (n=10)	98.8 ± 49.3	32.0 ± 9.2	181 ± 103	86.3 ± 26.7
1.56 mg/kg (n=10)	80.7 ± 13.8	29.7 ± 6.0	149 ± 59	84.2 ± 38.0
3.12 mg/kg (n=10)	95.9 ± 36.2	31.5 ± 10.5	170 ± 79	95.1 ± 44.8
6.25 mg/kg (n=9)	73.0 ± 11.7	24.9 ± 5.7	122 ± 59	70.7 ± 38.5

^aMPP = 4-nitrophenyl methyl (phenyl) phosphinate[†]Mean ± standard deviation[‡]Significantly higher than the dose groups (p < 0.05)

APPENDIX D (Cont)

TABLE 8

Effect of MPP* on Cholinesterase Activity in Plasma, Red Blood Cell and Brain

	(Acetyl) Cholinesterase Activity		
	Plasma I.U.	Red Blood Cell I.U.	Brain I.U.
MALES			
Untreated Controls (n=10)	$0.29 \pm 0.05^{\dagger}$	$1.81 \pm 0.19^{\dagger}$	9.16 ± 1.20
Vehicle Controls (n=10)	0.30 ± 0.06	$2.03 \pm 0.17^{\S}$	9.34 ± 1.05
2.12 mg/kg (n=9)	0.27 ± 0.04	1.60 ± 0.18	9.36 ± 1.18
4.25 mg/kg (n=9)	0.29 ± 0.04	1.60 ± 0.10	9.87 ± 1.62
8.50 mg/kg (n=9)	0.32 ± 0.07	1.58 ± 0.08	9.27 ± 0.74
17.00 mg/kg (n=1)	—	—	—
FEMALES			
Untreated Controls (n=10)	$1.16 \pm 0.34^{\text{II}}$	2.00 ± 0.17	9.73 ± 1.11
Vehicle Controls (n=9)	1.18 ± 0.42	$2.13 \pm 0.18^{\S}$	9.72 ± 1.41
0.78 mg/kg (n=10)	1.12 ± 0.47	1.74 ± 0.12	8.69 ± 2.78
1.56 mg/kg (n=10)	1.36 ± 0.47	1.73 ± 0.20	10.12 ± 1.46
3.12 mg/kg (n=10)	1.14 ± 0.41	1.80 ± 0.15	9.75 ± 1.46
6.25 mg/kg (n=9)	1.25 ± 0.41	1.72 ± 0.21	9.76 ± 1.50

* MPP = 4-nitrophenyl methyl (phenyl) phosphinate

[†] Mean \pm standard deviation

[‡] Significantly lower than the vehicle control group when compared by Student's t-test ($p < .05$)

[§] Significantly higher by analysis of variance with the dose groups ($p < 0.01$)

^{II} Significantly higher than cage control group for males ($p < 0.0001$)

APPENDIX D (concluded)

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HISTORICAL LISTING OF STUDY EVENTS

Dates		
Males	Females	Events
7 Jan 82	27 Jan 82	Rats arrived at LAIR, were examined for illness, ear tagged, weighed, and housed individually. Two animals were submitted to Pathology for quality control.
9 Jan 82	3 Feb 82	Rats were weighed.
11-15 Jan 82	1-5 Feb 82	Rats were predosed accclimated with 1 ml of water.
14 Jan 82	5 Feb 82	Rats were weighed and randomized into groups.
18-31 Jan 82	8-22 Feb 82	Groups 1 through 6 were dosed and observed daily.
18, 21, 25 28 Jan 82	8, 11, 15, 18 Feb 82	Groups 1 through 6 were weighed and dose calculated. Groups 7 through 12 were weighed.
21 Jan- 3 Feb 82	11-25 Feb 82	Groups 7 through 12 were dosed and observed daily.
21, 25, 28 Jan and 1 Feb 82	11, 15, 18, 22 Feb 82	Groups 7 through 12 were weighed and doses were calculated.
28 Jan 82	18 Feb 82	Groups 1 through 6 were tranferred to metabolic cages overnight for urine collection.
29 Jan 82	19 Feb 82	Groups 1 through 6 urine was collected and animals were returned to original cages.

APPENDIX E

Historical Listing of Study Events (Cont'd)

Dates		
Males	Females	Events
1 Feb 82	22 Feb 82	Groups 1 through 6 were weighed, observed, and submitted to Pathology for necropsy. Blood and tissue samples for analytical and clinical chemistry were taken during necropsy. Groups 7 through 12 were transferred to metabolic cages overnight for urine collection.
2 Feb 82	23 Feb 82	Groups 7 through 12 urine was collected and animals returned to original cages.
4 Feb 82	25 Feb 82	Groups 7 through 12 were weighed, observed, and submitted to pathology for necropsy. Blood and tissue samples for analytical and clinical chemistry were taken during necropsy.

APPENDIX E (Concluded)

PROCEDURES FOR ANALYTICAL CHEMISTRY

The following are GLP SOP's for the analytical chemistry performed for the study.

1. Calcium - OP-ACH-17
2. Potassium - OP-ACH-19
3. Chloride - OP-ACH-20
4. Glucose - OP-ACH-7
5. Cholesterol - OP-ACH-11
6. Creatinine - OP-ACH-15
7. Blood Urea Nitrogen - OP-ACH-16
8. Uric Acid - OP-ACH-14
9. Albumin - OP-ACH-12
10. Total Protein - OP-ACH-13
11. Aspartate Amino-Transferase - OP-ACH-4
12. Alanine Amino-Transferase - OP-ACH-3
13. Lactate Dehydrogenase - OP-ACH-5
14. Creatine Phosphokinase - OP-ACH-6
15. Acetyl Cholinesterase - OP-ACH-30 and OP-ACH-46

Serum magnesium was assayed by an adaptation of the Magnesium Rapid Stat^R Kit (Pierce Chemical Co., Rockford, IL 61105) to the GEMSAEC^R analyzer.

APPENDIX F

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CHANGES TO ORIGINAL OBJECTIVES AND PROCEDURES

1. Ear tags beginning with D8100- were used to identify rats since ear tags with D8200- numbers were not available.
2. The humidity stayed within the ranges specified under environmental conditions except on a few occasions. In the room with male rats, the humidity dropped to about 30% between 0800 and 1400 hours on 3 January 1982 and dropped to between 20 to 30% from 0800 hours on 4 January 1982 to 0800 hours on 5 January 1982. In the room with the female rats, the humidity increased to 60 to 90% between 0800 and 1800 hours on 6 February 1982 and increased to 70 to 85% from 2200 hours on 21 February 1982 to 0630 hours on 22 February 1982. No adverse effects were seen clinically or on necropsy due to these environmental fluctuations.
3. Male rats were randomized on 14 January 1982 instead of 15 January 1982 as stated in the original protocol dated 8 January 1982.
4. On 11 February 1982 observations for Groups 7 through 12 were not done. Group 11 observations were not conducted on 22 February 1982, and on 23 February 1982 observations were not conducted for Groups 7-12.
5. Animal D8100909 was removed after terminal sacrifice from the study due to a severe urinary tract infection. The infection was judged not to be compound related, consequently, the results for this animal from hematology and analytical chemistry were excluded from the statistical analysis of the data.
6. Animals that were misdosed and remained alive were not removed from the study, as is the practice with acute studies. Due to daily dosing, the chances of misdosing increased. Every group had at least one animal that was misdosed once. One male group and one female group contained three animals that were misdosed once. However, only two animals were misdosed more than once (D8100861 and D8100922). Usually when an animal is considered misdosed, the compound is injected into the esophagus instead of the stomach and appears in the mouth after removal of the needle. Some of the dose was probably lost, but it was assumed that more than half was ingested. Since this amount is small relative to what the animal would have received over the 14 days, this error was considered acceptable. Taking into consideration the crude methods of estimating the amount of compound received by an animal when it is placed in the diet, these deviations were judged to be negligible.

APPENDIX G

Lewis--38



DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULV-T

16 March 1983

MEMORANDUM FOR RECORD

SUBJECT: Statistical Analysis for GLP Studies 81034 and 81035

1. Five animals of each sex were first assigned to each of 12 groups using stratified sampling techniques via the TOXSYS[®] system. Then two groups were randomly assigned to each of the dose groups via the computer program, RANDOM.

2. The computer package, MINITAB, on the Data General MV/8000 computer was utilized to analyze the clinical chemistry and hematology data. Male rats (Study 81034) and female rats (Study 81035) were analyzed separately. The .05 level of significance was used in analyzing the data.

3. The measurements of the cage and vehicle control groups were compared using the Student's t-test.

a. Among the clinical chemistry data, no significant differences between the two groups were found in either sex for calcium, potassium, chloride, magnesium, plasma cholinesterase, brain acetylcholinesterase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine phosphokinase, glucose, cholesterol, blood urea nitrogen, uric acid, albumin, globulin and albumin/globulin ratio. Also, no differences were found between the two control group means in the females for creatinine, total protein and red blood cell acetylcholinesterase. The vehicle control group mean was found to be significantly greater than the cage control group mean in the males for creatinine, total protein and red blood cell acetylcholinesterase.

b. Among the hematology data, no significant differences between the two groups were found in either sex for red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count, white blood cell count, neutrophils, lymphocytes, eosinophils and monocytes. Also, no difference in females was found between these two groups for mean corpuscular volume measurement. However, in males, a significant difference between cage and vehicle control group means was found for the mean corpuscular volume.

4. One-way analysis of variance was used to test for differences between the four dose groups and the vehicle control group. When a significant F value for a group effect was found, a posteriori multiple comparisons were used to test for differences among means for the vehicle control group with a one-sided Dunnett's test.

SGRD-ULV-T

16 March 1983

SUBJECT: Statistical Analysis for GLP Studies 81034 and 81035

a. No differences in both sexes were found for the following clinical chemistry measurements: calcium, potassium, chloride, magnesium, plasma cholinesterase, brain acetylcholinesterase, alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, glucose, cholesterol, creatinine, blood urea nitrogen, uric acid, albumin, globulin, albumin/globulin ratio, and total protein. No difference was found in the male group means for lactate dehydrogenase. The female vehicle control group mean was found to be significantly greater than the female dose group means for lactate dehydrogenase. All dose groups for both sexes were found to have group means which are significantly less than the vehicle control group mean for red blood cell acetylcholinesterase.

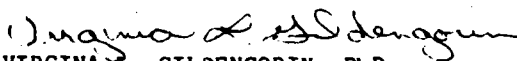
b. No differences in both sexes were found for the following hematological measurements: mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, reticulocyte count, white blood cell count, neutrophils, lymphocytes, eosinophils and monocytes. Also no difference was found in the group means in males for red blood cell count, hemoglobin, hematocrit and platelets. The female vehicle control group mean was found to be significantly greater than the highest female dose group (6.25 mg/kg) mean for red blood cell count, hemoglobin, hematocrit and platelet count. The female vehicle control group (0.78 mg/kg) mean was found also to be significantly higher than the lowest female dose group mean for platelets.

5. The Bliss Method of probit analysis was performed on the percent of deaths in each of the male dose groups using the program, PROBIT. The regression line determined for the analysis is:

$$Y = .204 + 4.598 \text{ Log } (X)$$

where X is the dose and Y is its corresponding probit. The program transforms the predicted probits to obtain LD₁, LD₅₀, and LD₉₅ estimates along with their corresponding 95% fiducial limits.

6. The time of survival data of the male rats in the highest dose group was examined by Cutler-Ederer's life table method, to yield rough estimates of the 25%, 50% and 75% survival times from a cumulative proportion survival function.


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Pathology Report

Fourteen Day Sub-chronic Toxicity Study of 4-Nitrophenyl
Methyl(phenyl)phosphinate in Male Albino Sprague-Dawley Rats, Study
81-0341. Introduction.

The objective of this study was to determine the sub-chronic effects of orally administered (gavage) 4-Nitrophenyl Methyl(phenyl)phosphinate in male Sprague-Dawley rats. The rats were predosed with water and then were divided randomly into 6 dose level groups of 10 animals each. The dosage levels of male rats were as follows:

Cage controls - groups 3 & 9
Vehicle* controls - groups 4 & 7
17 mg/kg - groups 2 & 8
8.5 mg/kg - groups 1 & 11
4.25 mg/kg - groups 6 & 12
2.12 mg/kg - groups 5 & 10

Urine samples were collected from individual rats in metabolic cages on the day before scheduled necropsy. A routine and microscopic urinalysis was performed on each sample to include: color, specific gravity, urobilinogen, occult blood, bile, ketones, glucose, protein, pH, white blood cells, red blood cells, epithelial cells, white blood cell casts, red blood cell casts, hyaline casts, granular casts, bacteria, crystals, mucus, and nitrite. After 14 days on test, the rats were submitted for necropsy. Following anesthesia with pentobarbital sodium, administered by intraperitoneal injection, blood was collected from the right ventricle of each rat and submitted for hematologic examination [red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), WBC differential and blood cell morphology, platelet count, and reticulocyte count]. Additional blood was submitted to Analytical Chemistry Services Group, Division of Research Support, for chemical analyses. All rats were killed by exsanguination and gross necropsy examinations were performed. Portions of anterior cerebrum (unfixed) were submitted to Analytical Chemistry Services Group, Division of Research Support, for cholinesterase determinations. Tissue specimens from major organs and systems were fixed in 10% neutral buffered formalin for subsequent microscopic examination. Tissues were embedded in paraffin, sectioned at approximately 6 microns thickness and stained with hematoxylin and

*Vehicle: 21.5% Polysorbate 80 (Tween 80), 18.5% Ethanol, 37.5% Citrate Buffer, 21.5% distilled water, 1.0% fructose.

eosin. All tissues itemized in SOP CP PSG 12 were examined microscopically in the cage controls, vehicle controls, and the 17 mg/kg dosage level. In the 8.5 mg/kg, 4.25 mg/kg, and 2.12 mg/kg dosage levels, only hearts, lungs, livers, and kidneys were examined microscopically.

2. Results, interpretation, and discussion.

The gross and/or microscopic findings are itemized in Incidence Tables 1 - 2.†

a. Table 1 tabulates the incidence and severity of lesions observed grossly or microscopically in each rat.

b. Table 2 tabulates the microscopic lesions observed in the rats utilized in the study at each dosage level.

c. Clinical pathology: One-way analysis of variance was performed on WBCs, white cell differentials, RBCs, MCVs, MCHs, MCHCs, hemoglobin concentrations, Hcts, reticulocyte counts and platelet counts to determine if there were any differences between the 6 groups of control and test rats. In all but one case the "P-value" was greater than 0.05. The MCV determination was significantly less in the vehicle control group than in the cage control group.

Urine samples were collected in metabolic cages. Due to fecal contamination and dilution from water bottle spillage, statistical analysis of the urinalysis data was not performed.

d. Gross necropsy:

The deaths of 8/9** male rats at the 17 mg/kg dosage level, 1/10 at the 8.5 mg/kg level, and 1/10 at the 4.25 mg/kg level were attributed to the toxic effect of the tested compound. All of the compound related deaths were observed on the fourth through the twelfth day of the study. Three rats at the 17 mg/kg level, that were included in the deaths due to the toxic effect of the compound, were actually killed when moribund. An additional rat at the 17 mg/kg level died on day 2 of the study and was deleted from the death count because it had a perforated trachea. However, this rat will be included at the 17 mg/kg dosage level in the discussion of both the gross and microscopic lesions. Ten out of ten cage controls, 10/10 vehicle controls, and 10/10 at the 2.12 mg/kg dosage level survived until termination of the study.

**Number of rats affected/number of rats in dosage level.

† Archived with raw data.

Gross changes attributable to aspiration of water, test compound, vehicle, and/or fluid gastric contents were present in rats that received test compound at all dosage levels. One or more red to brown foci were observed in the lungs of 5/10 rats at the 17 mg/kg level, 3/10 at the 8.5 mg/kg level, 2/10 at the 4.25 mg/kg level, and 3/10 at the 2.12 mg/kg level. A portion of at least one lung lobe was red to plum colored or wet in 2/10 rats at the 17 mg/kg level, 1/10 at the 8.5 mg/kg level, and 1/10 at the 4.25 mg/kg level. These lung lesions probably represent congestion, hemorrhage, and/or pneumonia.

Gross changes were present in the digestive system of 2/10 rats at the 17 mg/kg dosage level and 1/10 at the 8.5 mg/kg level that may have been due to an irritant effect of the test compound. The small intestines of all three contained yellow to red mucoid material. The stomachs of both rats at the 17 mg/kg level had one or more red, brown, or black mucosal foci and the stomach of one rat contained yellow mucoid material. The colon of one rat at the 17 mg/kg level contained yellow mucoid material.

Oily to crusty material was observed on the hair of the muzzle and/or forepaws of 5/10 rats at the 17 mg/kg dosage level. Yellow mucoid material was present in the esophagus of a single rat at the 4.25 mg/kg dosage level. These findings suggest the reflux and aspiration of tested compound, vehicle, and/or stomach contents.

A few lesions were observed that were not test compound related. These consisted of dilated renal pelves, partially retained testes, gas filled stomach and small intestine, fractured digit, congenital anomaly (situs inversus) of lung, splenic nodule, brown material on the prepuce, and a dermal abrasion on the neck.

e. Microscopic findings and conclusions:

All microscopic lesions observed in the male Sprague-Dawley rats on this study that were considered to be due to agent, vehicle, and/or dosing technique were restricted to the lungs, with the possible exceptions of esophagitis and gastritis in separate rats at the the 17 mg/kg dosage level. The pulmonary lesions observed in rats at the various dosage levels occurred as follows:

(1) Subacute pneumonia was observed in 2/10 vehicle controls, 4/10 cage controls, 6/10 at the 17 mg/kg dosage level, 6/10 at the 8.5 mg/kg dosage level, 2/10 at the 4.25 mg/kg dosage level, and 3/10 at the 2.12 mg/kg dosage level.

(2) Chronic pneumonia was observed in 1/10 rats at the 4.25 mg/kg dosage level.

(3) Subacute pleuritis was observed in 1/10 vehicle controls.

(4) Acute hemorrhage was observed in 2/10 vehicle controls, 1/10 cage controls, 3/10 at the 17 mg/kg dosage level, 3/10 at the 8.5 mg/kg dosage level, 3/10 at the 4.25 mg/kg dosage level, and 2/10 at the 2.12 mg/kg dosage level.

(5) Hemoglobin crystals were observed in 1/10 rats at the 17 mg/kg dosage level, 2/10 at the 8.5 mg/kg dosage level, and 1/10 at the 2.12 mg/kg dosage level.

(6) Pigment laden macrophages were observed in 1/10 vehicle controls.

(7) Bronchitis/bronchiolitis was observed in 2/10 cage controls, 3/10 at the 17 mg/kg dosage level, 1/10 at the 8.5 mg/kg dosage level, and 2/10 at the 4.25 mg/kg dosage level.

Most likely the pulmonary lesions itemized above were due to the aspiration of water, vehicle, test compound, and/or fluid gastric contents as a result of the gastric intubation. The esophagitis was most likely the result of laceration by the stomach tube. The necrotizing gastritis may have been due to the irritant effect of the test compound.

The following lesions were observed during microscopic examination of tissue from male Sprague-Dawley rats on this study and were not considered to be caused by tested compound, vehicle, or dosing technique: tracheitis, mononuclear infiltrate in exorbital lacrimal glands, dacryoadenitis and mononuclear infiltrate in Harderian glands, epicarditis and mononuclear infiltrate in hearts, foamy alveolar macrophages and lymphoid hyperplasia in the lungs, hepatitis in livers, nephritis, mononuclear infiltrate, dilated tubules, concretions, and dilated renal pelvis in kidneys, prostatitis and mononuclear infiltrates in prostates, inflammation of accessory sex glands, hemorrhage in skeletal muscle at the base of the skull, and rhinitis. These lesions occurred with great variability or with approximately equal frequency in controls and rats dosed with test compound. In a few instances the lesions occurred only in or with slightly increased incidence in either the controls or the rats dosed with test compound. All of these lesions have spontaneously occurred, on occasion, in our laboratory.

3. Summary.

a. The deaths of 8/9 male Sprague-Dawley rats at the 17 mg/kg dosage level, 1/10 at the 8.5 mg/kg dosage level, and 1/10 at the 4.25

mg/kg dosage level were attributed to the toxic effect of the tested compound and indicates a dose related response.

b. Gross pulmonary lesions were observed in one or more rats from each dosage level that received tested compound and represented congestion, hemorrhage, and/or pneumonia. These changes were attributed to the aspiration of water, tested compound, vehicle, and/or fluid gastric contents.

c. Gross changes were present in the digestive system in one or more rats receiving test compound at the two highest dosage levels. The only microscopic lesion observed in the digestive system was an acute gastritis in one rat at the 17 mg/kg dosage level. These changes may have been due to an irritant effect of the tested compound.

d. Microscopic lesions were observed in the lungs of Sprague-Dawley rats at all dosage levels (to include cage and vehicle controls). These lesions were most likely due to the aspiration of water, vehicle, tested compound, and/or fluid gastric contents as a result of the gastric intubation.

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Pathology Report

Fourteen Day Sub-chronic Toxicity Study of 4-Nitrophenyl
Methyl(phenyl)phosphinate in Female Albino Sprague-Dawley Rats,
Study 81-035

1. Introduction.

The objective of this study was to determine the sub-chronic effects of orally administered (gavage) 4-Nitrophenyl Methyl(phenyl)phosphinate in female Sprague-Dawley rats. The rats were predosed with water and then were divided randomly into 6 dose level groups of 10 animals each. The dosage levels of female rats were as follows:

Cage controls - groups 1 & 11
Vehicle* controls - groups 5 & 12
6.25 mg/kg - groups 3 & 10
3.12 mg/kg - groups 2 & 9
1.56 mg/kg - groups 4 & 7
0.78 mg/kg - groups 6 & 8

Urine samples were collected from individual rats in metabolic cages on the day before scheduled necropsy. Following anesthesia with pentobarbital sodium, administered by intraperitoneal injection, blood was collected from the right ventricle of each rat and submitted for hematologic examination [red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), WBC differential and blood morphology, platelet count, and reticulocyte count]. Additional blood was submitted to Analytical Chemistry Services Group, Division of Research Support, for chemical analysis. All rats were killed by exsanguination and complete gross necropsy examinations were performed. Portions of anterior cerebrum (unfixed) were submitted to Analytical Chemistry Services Group, Division of Research Support, for cholinesterase determinations. Tissue specimens from all major organs and systems were fixed in 10% neutral buffered formalin for subsequent microscopic examination. Tissues were embedded in paraffin, sectioned at approximately 6 microns thickness and stained with hematoxylin and eosin. All tissues itemized in SOP OP PSG 13 were examined microscopically in the cage controls, vehicle controls, and the 6.25 mg/kg dosage level. In the 3.12 mg/kg, 1.56 mg/kg, and 0.78 mg/kg dosage levels, only hearts, lungs, livers, and kidneys were examined microscopically.

*Vehicle: 21.5% Polysorbate 80 (Tween 80), 18.5% Ethanol, 37.5% Citrate Buffer, 21.5% distilled water, 1.0% fructose.

APPENDIX I (cont.)

2. Results, interpretation, and discussion.

The gross and/or microscopic findings are itemized in Incidence Tables 1 and 2.[†]

a. Table 1 tabulates the incidence and severity of lesions observed grossly and/or microscopically in the tissues of each rat.

b. Table 2 tabulates the microscopic lesions observed in all of the rats utilized in the study at each dosage level.

c. Clinical pathology:

Red blood cell counts, hemoglobin, hematocrit, and platelet counts were significantly lower in rats in the high dose group (6.25 mg/kg) compared to those in the vehicle control group. There were no differences in these values between vehicle control and cage control groups. (See Statistician's Report, Appendix C). Platelet counts were also lower in the .78 mg/kg dose group. No differences between group means were found for WBC, MCV, MCH, MCHC, reticulocyte count or white blood cell differential values.

The differences observed in the high dose group indicate that, as a group, these animals had mild normocytic, normochromic anemia compared to controls. No gross or microscopic lesions were observed in hematologic organs of these animals. The decrease in these measurements could be caused by either acute blood loss or possibly increased rate of destruction or decreased rate of formation of erythrocytes. No evidence of hemorrhage was observed grossly or microscopically except for very mild lesions in the lungs which occurred with approximately the same frequency and intensity in all groups. The occurrence of significant hematologic changes in the high dose group suggests that they are an effect of the tested compound. However, the mechanism(s) by which the compound causes these effects is unknown.

In future toxicologic tests of this compound, special attention should be given to evaluation of blood and hematologic tissues to determine whether or not these tissues are affected and which mechanisms are involved.

Urine specimens, collected in metabolic cages, were unsuitable for examination due to fecal contamination and spillage from water bottles. For these reasons no attempts to evaluate urinalysis data were made.

d. Gross necropsy:

There were no deaths in any of the dosage groups that were attributable to the toxic effects of the test compound. One rat in the 6.25 mg/kg level died during the course of the study. The death in this case was attributed

[†] Archived with raw data.

to trauma caused during the course of dosing. Although a tear in the trachea was not found, anterior mediastinal emphysema indicated a rupture somewhere in the upper respiratory tree.

Gross changes attributable to aspiration of water, test compound, vehicle, and/or fluid gastric contents were present in rats that received test compound in all groups. One or more red to brown foci were observed in the lungs of 6/10 rats in the vehicle control group, 4/10 rats in the cage control group, 4/10 rats at the 6.25 mg/kg level, 5/10 rats at the 3.12 mg/kg level, 6/10 rats at the 1.56 mg/kg level, and in 4/10 rats at the 0.78 mg/kg level. Depressed foci of the lungs were seen in 1/10 rats in the vehicle control group, 1/10 rats in the cage control group, and 1/10 rats at the 6.25 mg/kg dosage level. Mediastinal emphysema was seen in 1/10 rats at the 6.25 mg/kg level. Fibrinous adhesions and hydrothorax were seen in 1/10 rats at the 0.78 mg/kg level. These lesions probably represent congestion, hemorrhage, and/or pneumonia.

Gross changes were seen in the kidneys of 1/10 rats at the 6.25 mg/kg level, in 1/10 rats at the 1.56 mg/kg level, and in 1/10 rats at the 0.78 mg/kg level. The gross lesion in all of these rats consisted of dilation of either right or left or both renal pelves. This change is not considered to be compound related.

Mucoid, red-yellow, material was found in the small intestine of 1/10 rats in the 6.25 mg/kg level. This change was observed only in the rat that died during the study. This change may have been an irritant effect of the test compound.

White calculi were found in the urinary bladder of 1/10 rats in the vehicle control group.

e. Microscopic findings and conclusions:

All microscopic lesions observed in female Sprague-Dawley rats on this study that were considered to be due to agent, vehicle, and/or dosing technique were restricted to the lungs. The pulmonary lesions observed in rats at the various dosage levels occurred as follows:

(1) Subacute pneumonia was observed in 2/10 vehicle control rats, 4/10 cage control rats, 8/10 at the 6.25 mg/kg dosage level, 2/10 rats at the 3.12 mg/kg dosage level, 5/10 rats at the 1.56 mg/kg dosage level, and 4/10 rats at the 0.78 mg/kg dosage level.

(2) Chronic pneumonia was observed in 1/10 rats in the vehicle control group and in 1/10 rats in the cage control group.

(3) Subacute pleuritis was observed in 1/10 rats at the 0.73 mg/kg dosage level.

(4) Acute hemorrhage was observed in 3/10 vehicle controls, 1/10 cage controls, 3/10 rats at the 6.25 mg/kg dosage level, 6/10 at the 3.12 mg/kg dosage level, 3/10 at the 1.56 mg/kg dosage level, and 3/10 at the 0.78 mg/kg dosage level.

(5) Bronchitis/bronchiolitis was observed in 2/10 vehicle controls, 4/10 cage controls, 1/10 rats at the 6.25 mg/kg dosage level, 3/10 rats at the 3.12 mg/kg dosage level, and 2/10 rats at the 1.56 mg/kg dosage level, and 2/10 rats at the 0.78 mg/kg level.

(6) Lymphoid hyperplasia was observed in 5/10 cage controls, 1/10 rats at the 3.12 mg/kg dosage level, 2/10 rats at the 1.56 mg/kg dosage level, and 2/10 rats at the 0.78 mg/kg dosage level.

Most likely the pulmonary lesions itemized above were due to the aspiration of water, vehicle, test compound, and/or fluid gastric contents as a result of the gastric intubation.

The following lesions were observed during microscopic examination of tissue from female Sprague-Dawley rats on this study and were not considered to be caused by test compound, vehicle, or dosing technique: subacute tracheitis, subacute sialoadenitis, subacute dacryoadenitis, subacute epicarditis, mononuclear infiltrate of the myocardium, parathyroid inflammation, subacute hepatitis, mononuclear infiltrate of the liver, interstitial nephritis, mononuclear infiltrate of the kidney, intratubular concretions in the kidney, subacute pyelitis, dilated renal pelvis, subacute cystitis, lobular atrophy of the pancreas, subacute rhinitis, and purulent otitis media. These lesions occurred with great variability or with approximately equal frequency in controls and rats dosed with test compound. In a few instances the lesions occurred only in or with slightly increased incidence in either the controls or the rats dosed with test compound. All of these lesions have occurred spontaneously on occasion, in our laboratory.

3. Summary.

a. No compound related deaths occurred during this study.

b. Statistically significant reductions in RBC, Hb, HCT, and platelet counts occurred in the high dose group (6.25 mg/kg). These changes in hematologic values may be due to the tested compound.

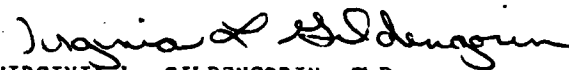
c. Gross pulmonary lesions were observed in one or more rats from each dosage level that received test compound and consisted of congestion, hemorrhage, and/or pneumonia. These changes were attributed to the

aspiration of water, test compound, vehicle, and/or fluid gastric contents.

d. Microscopic lesions were observed in the lungs of Sprague-Dawley rats at all dosage levels (including cage and vehicle controls). These lesions were most likely due to the aspiration of water, vehicle, test compound, and/or fluid gastric contents as a result of the gastric intubation.



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